

Impacts of Coalmine Discharges on Illinois Unionid Mussels

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Introduction

While active coalmines are subject to National Pollutant Discharge Elimination System (NPDES) permits requiring periodic monitoring of the chemical content and/or toxicity of discharges, little is known about the extent to which these monitoring techniques are protective of indigenous freshwater mussels (Unionidae). As an example, Belanger et al. (1990) noted that only one laboratory study of acute copper toxicity or chronic impairment to freshwater bivalves was included in the United States Environmental Protection Agency's (U.S. EPA, 1985) ambient water quality criteria document for Cu, and those data were not used in criteria calculation. Cherry et al. (2002) recently conducted a study to determine if effluent permit limits for copper at a coal-fired power plant located on the Clinch River, Virginia, were protective of native unionids. They tested the toxicity of Cu to 17 genera, including 15 that were indigenous to the Clinch River, as well as the standard U.S. EPA effluent testing organisms, *Ceriodaphnia dubia* (a cladoceran) and *Pimephales promelas* (the fathead minnow). Seven of the 10 most sensitive genera to copper in this study were freshwater mussels, including the top three. *C. dubia* ranked sixth, after four mussels and a mayfly, and the fathead minnow ranked fourteenth. This study provided evidence that mussels may be among the most sensitive aquatic organisms to contaminants, and that standard bioassessment techniques (e.g., water column toxicity testing with *C. dubia*) and WQC standards may not be protective of them.

The objective of this study was to conduct various bioassessment techniques to determine if the discharge from the Riola Mine was causing measurable impairment to instream communities, particularly to mussels.

Methods

Water/Sediment Chemistry

Water chemistry analysis included general wet chemistry (temperature, pH, dissolved oxygen, conductivity, alkalinity and hardness) and metals analysis. Temperature, pH, dissolved oxygen, and conductivity were measured in the field, while alkalinity and hardness (mg/L as CaCO₃) were determined in the laboratory by titration (APHA, 1998). Water samples were analyzed for total concentrations of Al, Ca, Cd, Cu, Fe, magnesium (Mg), manganese (Mn), Na, lead (Pb), and zinc (Zn) by Inductively-Coupled Plasma (ICP) spectrometry at the Illinois State Water Survey, Champaign, IL.

Laboratory Water Column Toxicity Testing

Water column samples will be collected from each sampling station to test for toxicity to the cladoceran, *Ceriodaphnia dubia*. *C. dubia* has been cultured house according to U.S. EPA methods (1993) in the Soucek laboratory at the Illinois Natural History Survey since 2002. Prior to testing, organisms were fed a diet of *Selenastrum capricornutum* and a Yeast-Cereal Leaves-Trout Chow (YCT) mixture at a rate of 0.18 ml each per 30-ml water, daily. Cultures were maintained at $25\pm 1^{\circ}\text{C}$, and a 16:8 (L:D) photoperiod.

Range finding tests were conducted for each site (upstream and downstream of discharge) to determine if acute or chronic toxicity testing was appropriate. For range finding tests, five organisms were placed into each of four replicate 50-ml beakers containing undiluted water collected from each station for 48 h. Moderately hard, reconstituted (EPA¹⁰⁰) water was used as the control. At the end of the test period, percent survival for each station was recorded. Results of range finding tests indicated that further testing (i.e., generation of LC50s) was not required.

Laboratory Sediment Toxicity Testing

Whole-sediment samples were collected from each site according to standard methods (U.S. EPA, 2001) using a polyethylene scoop. Whole sediments were tested for toxicity to the midge, *Chironomus tentans*, according to U.S. EPA methods (1994

Benthic Macroinvertebrate Community Sampling

Benthic macroinvertebrate surveys were conducted concurrent with the mussel transplant test according to the U.S. EPA Rapid Bioassessment Protocols (RBP) with slight modifications (Barbour et al., 1999). Modifications of RBP protocols included the fact that riffle, run, pool and shoreline rooted areas were sampled for 5 minutes per site using dip-nets with 800- μm mesh dipnets rather than for 20 “jabs” with a 500- μm mesh dipnet. One composite sample was collected per site according to RBP recommendations. All organisms in each sample were identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins, 1996; Pennak, 1989). Chironomids were identified as either subfamily Tanypodinae or non-Tanypodinae.

Caged Transplanted Mussel Study

To experimentally evaluate the toxicity of mining discharges to freshwater mussels, fatmuckets (*Lampsilis siliquoidea*) were collected from a nearby reference site within the same drainage (Little Vermillion River at CR 600 E, north of Sidell) for transplant studies. Organisms were collected, transported to the laboratory, sexed, measured for shell length (anterior to posterior margin) and depth (dorsal to ventral margin) and wet mass. Mussels then were secured in plastic cages, which were subdivided and marked to allow identification of individuals before and after deployment. Approximately 2 days after collection of mussels, cages were transported back to the field sites and secured to the substrate using re-bar. Five cages were placed at each monitoring station in Fayette Drain (upstream and downstream of the Riola mine property) and each cage contained one male and one female mussel. Cages were

monitored on a weekly basis to prevent filling with sediments. After 60 days, cages were removed from the sampling stations, organisms were transported back to the laboratory, and shell length, wet mass, tissue condition index (dry tissue mass divided by dry shell mass, Newton et al., 2001). Dried soft tissues were digested for metals analysis according to U.S. EPA (1991) methods, and tissue digestates were submitted to the Illinois State Water Survey for analysis using ICP spectrometry. Tissues from both mussels in a given cage were combined for digestion and analysis, resulting in five mussel tissue samples per sampling station.

RESULTS

Riola Mine Discharge, Fayette Drain, near Georgetown, IL.

According to IDNR staff, the Black Beauty Riola Coal underground coalmine, located in Vermilion County, with both open and reclaimed gob/caked slurry coal waste facilities was opened in 1994 on the Fayette Drain. Fayette Drain is a tributary to the Little Vermilion River, which is known to support populations of the State-listed endangered Little Spectaclecase, *Villosa lienosa*, and the State-threatened Slippershell, *Alasmodonta viridis*. An IDNR survey crew in August 2001 found 18 *V. lienosa* and 2 *A. viridis*, along with individuals of four other species, including the formerly State-listed Pondhorn, *Unio merus tetralasmus*, less than one mile downstream of the Riola Mine outfall within a channelized portion of the stream. Listed species comprised 80% of live mussels found. The Riola Mine received a modified NPDES permit in late 2001.

During a site visit on September 2, 2002, a sample of the discharge was collected and analyzed for metals and general water chemistry. General water chemistry was as follows: pH 7.5, conductivity 1,450 $\mu\text{mhos/cm}$, alkalinity 100 mg/L (as CaCO_3), hardness 230 mg/L (as CaCO_3). This discharge sample was analyzed for total metal concentrations by inductively coupled plasma spectrophotometry at the Illinois State Water Survey. These concentrations are shown in Table 1. Eight priority toxic pollutants with Water Quality Criteria (WQC) for protection of aquatic life (U.S. EPA, 1999) were measured (beryllium, cadmium, chromium, copper, lead, nickel, selenium, and zinc) and with the exception of zinc, all were below detection limits. Zinc was substantially below its WQC limit of 0.240 mg/L (at hardness of 230 mg/L as CaCO_3). Five non-priority pollutant metals (U.S. EPA, 1999) were analyzed (aluminum, barium, boron, iron, and manganese), all with concentrations below respective WQC for protection of aquatic life or human health. **The discharge was not flowing during other site visits.**

Table 1. Total metal concentrations in water sample collected from the Black Beauty Riola Mine discharge into the Fayette Drain, Vermillion County, IL. The sample was collected directly from the discharge pipe, not from the stream. BDL = below detection limit. All concentrations in mg/L (ppm) total metal.

metal	concentration	metal	concentration
Al	0.063	Mo	0.009
B	0.296	Na	378
Ba	0.083	Ni	bdl
Be	bdl	P	bdl
Ca	61.8	Pb	bdl
Cd	bdl	S	92.1
Co	bdl	Sb	0.029
Cr	bdl	Se	bdl
Cu	bdl	Si	0.869
Fe	0.024	Sn	bdl
K	6.65	Sr	0.183
Li	0.019	V	0.019
Mg	18.0	Zn	0.001
Mn	0.005		

Laboratory Water Column and Sediment Toxicity Testing

Water samples collected from upstream and downstream reaches, and from the discharge itself caused no acute toxicity to *Ceriodaphnia dubia* on any occasion. In fact no test organisms died at all as a result of exposure to collected water column samples. Likewise with whole sediment toxicity tests with *Chironomus tentans*, samples collected from upstream and downstream sites did not cause significant mortality or growth reduction to the midge larvae.

Benthic Macroinvertebrate Community Sampling

A total of 2,562 individuals belonging to 36 different taxa were collected at both sites (Table 2). At the upstream site, 24 taxa were identified, while 36 were identified at the downstream site. The upstream site was dominated by dipterans (mostly of the family Chironomidae) and hemipterans (Corixidae), whereas the downstream site was dominated by both gastropod and bivalved (Pelecypoda) mollusks (Fig. 1). Two different genera of bivalves were found at the downstream site: *Pisidium* (252 specimens) and *Sphaerium* (22 specimens), both fingernail clams. Only four individuals of the genus *Pisidium* were found at the upstream site. Each site had the same three genera of mayflies (*Caenis* sp., *Hexagenia* sp., and *Baetis* sp.), but 113 mayflies were collected at the downstream site whereas only 31 were collected upstream of the discharge.

Table 2. Complete list of organisms collected using RBP methods both upstream and downstream of the Riola Mine discharge site. Yellow shading indicates a taxon not present at one site but collected at the other.

			upstream	downstream
Family	Taxon	common name	# of specimens	# of specimens
Annelids				
	Hirudinea	leech	1	0
	Oligochaeta	segmented worm	78	28
Mollusks				
Pelecypoda/Veneroida				
Sphaeriidae	<i>Pisidium</i>	fingernail clam	4	252
Sphaeriidae	<i>Sphaerium</i>	fingernail clam	0	22
Gastropoda				
Lymnaeidae	<i>Fossaria</i>	snail	1	100
Lymnaeidae	<i>Pseudosuccinea</i>	snail	0	1
Physidae	<i>Physella</i>	snail	133	334
Planorbidae	Planorbidae	snail	0	3
Crustaceans				
Malacostraca/Decapoda				
Cambaridae	<i>Orconectes propinquus</i>	crayfish	16	21
Cambaridae	<i>Procambarus acutus</i>	crayfish	4	16
Malacostraca/Amphipoda				
Hyalellidae	<i>Hyalella azteca</i>	scud (amphipod)	1	2
Malacostraca/Isopoda				
Asellidae	Caecidotea	aquatic sowbug (isopod)	91	9
Insects				
Ephemeroptera				
Caenidae	<i>Caenis</i>	square-gilled mayfly	19	86
Ephemeridae	<i>Hexagenia</i>	burrowing mayfly	11	1
Baetidae	<i>Baetis</i>	swimming mayfly	1	26
Odonata				
Calopterygidae	<i>Calopteryx</i>	damselfly	6	5
Coenagrionidae	<i>Enallagma</i>	damselfly	8	16
Gomphidae	<i>Dromogomphus</i>	dragonfly	1	7
Libellulidae	<i>Plathemis lydia</i>	dragonfly	0	11
Hemiptera				
Corixidae	juveniles	water boatman	235	38
Gerridae		water strider	0	1
Trichoptera				
Leptoceridae	<i>Oecetis</i>	caddisfly	0	2
Lepidoptera				
Pyalidae	<i>Acentria</i>	aquatic moth	0	1
Coleoptera				
Haliplidae	<i>Peltodytes</i>	crawling water beetle	13	70
Elmidae	<i>Dubiraphia</i>	riffle beetle	56	26

Gyrinidae	<i>Gyrinus</i>	whirligig beetle	0	3
Hydrophilidae	<i>Tropisternus</i>	water scavenger beetle	1	3
Dytiscidae	<i>Agabus</i>	predaceous diving beetle	2	17
Dytiscidae	<i>Laccophilus</i>	predaceous diving beetle	0	2
Dytiscidae	<i>Rhantus</i>	predaceous diving beetle	2	0
Dytiscidae	<i>Derovatellus</i>	predaceous diving beetle	0	4
Diptera				
Tabanidae	<i>Chrysops</i>	deer fly	1	7
Ceratopogonidae	<i>Bezzia</i>	No-see-um or biting midge	15	3
Chironomidae		non-biting midge	657	81
Simuliidae		black fly	0	2
Stratiomyidae		soldier fly	0	1
Sciomyzidae		marsh fly	0	4
	total abundance		1357	1205
	total richness		24	35
	EPT richness		3	4

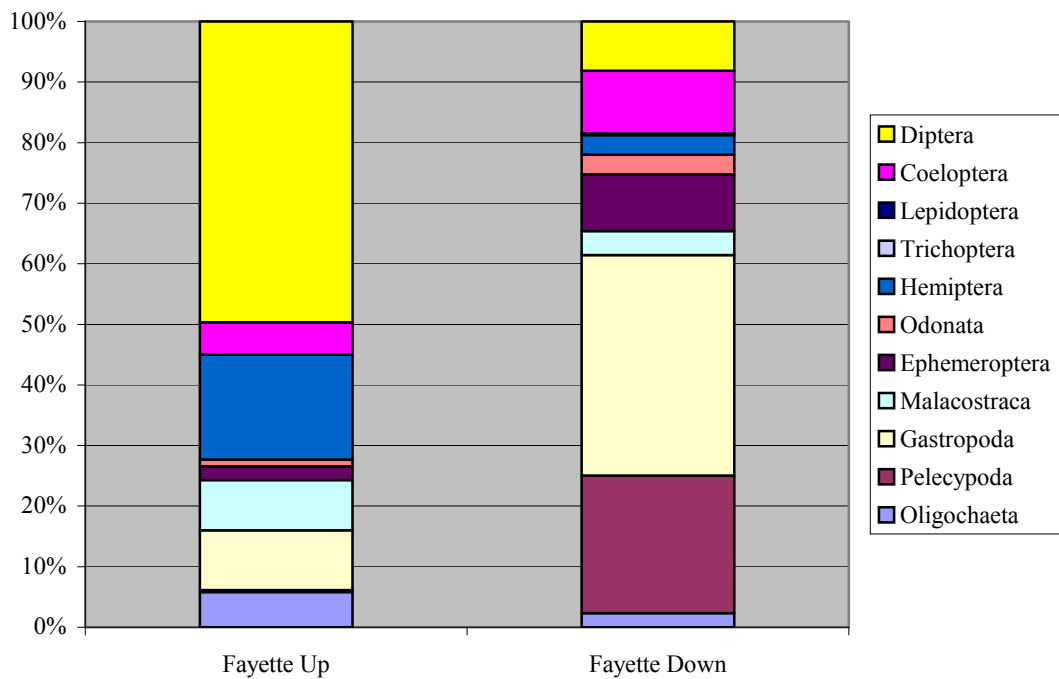


Figure 1. Taxonomic composition of benthic communities at sampling sites upstream and downstream of the Riola Mine discharge point in Fayette Drain. Bars show percent abundance of various taxa, e.g. the bright yellow portion of each bar indicates the percentage of the total number of organisms collected that belong to the insect Order Diptera.

Caged Transplanted Mussel Study

At the end of 60 days, mussels caged and transplanted to sites upstream and downstream of the Riola mine discharge were evaluated for fitness using various body measurements (Table 3). Mussels were similar in whole body weight, length (anterior to posterior margin), depth (dorsal to ventral margin), dry tissue weight and shell weight when comparing means of all mussels at a given site. That is, the average values of these parameters for upstream mussels were not significantly different ($p > 0.05$) from those for mussels transplanted downstream of the discharge. However, despite the fact that organisms collected from the Little Vermillion River were selected from a specific size range, variability in these parameters before transplantation may reduce the ability to statistically detect differences due to the discharge. Therefore, an additional normalizing parameter called Tissue Condition Index (TCI) was calculated. This index is a measure of the relative fitness of the organism because it divides the mass of the dried soft parts of the organism by the mass of the dry shell, i.e., it is a measure of the robustness of the organisms. This normalized parameter is more appropriate for comparing fitness of organisms upstream and downstream of the discharge. As shown in table 2, the mean TCI value of the mussels at the downstream site was nominally lower than that for the upstream mussels (higher TCI suggests better condition); however, these means were not significantly different ($p = 0.2373$). Therefore, it appears that site of transplantation had no effect on fitness of the caged mussels.

Table 3. Various body measurements of mussels (*Lampsilis siliquioidea*) after being transplanted upstream and downstream of the Riola mine discharge in Fayette Drain for 60 days. Individual measurements and averages are shown.

Upstream						
Individual	Weight (mm)	Length (mm)	Depth (mm)	Dry Tissue Weight	Shell Weight	TCI
1	90.38	80.52	48.68	2.06	51.18	4.02
2	94.59	84.85	48.92	2.15	46.47	4.62
3	110.02	83.90	53.01	2.72	57.09	4.77
4	99.87	90.52	53.38	2.65	48.69	5.44
5	96.01	83.78	52.11	2.76	51.54	5.36
6	89.43	84.41	51.39	2.46	43.80	5.61
7	102.45	85.20	51.39	2.84	50.65	5.62
8	96.90	86.94	51.32	3.04	48.76	6.24
9	80.49	77.91	49.49	1.83	41.81	4.37
10	77.31	83.57	49.02	2.60	38.35	6.77
average	93.74	84.16	50.87	2.51	47.83	5.28
std dev	9.83	3.38	1.73	0.39	5.42	0.85
Downstream						
Individual	Weight (mm)	Length (mm)	Depth (mm)	Dry Tissue Weight	Shell Weight	TCI

1	124.74	89.81	53.20	2.63	62.35	4.21
2	113.88	88.58	50.81	2.73	61.94	4.40
3	96.69	83.48	54.25	1.70	49.04	3.47
4	96.87	86.87	50.53	2.64	49.29	5.36
5	76.23	79.53	48.11	2.05	39.01	5.24
6	79.29	86.75	48.31	2.32	39.23	5.92
7	92.58	78.29	51.03	2.24	48.99	4.57
8	92.60	90.66	53.98	2.55	45.77	5.56
9	dead	n/a	n/a	n/a	n/a	n/a
10	94.98	85.27	51.14	2.29	48.70	4.70
average	96.43	85.47	51.26	2.35	49.37	4.83
std dev	15.16	4.33	2.22	0.33	8.29	0.76

Generally, elemental concentrations in dried mussel tissues were similar for organisms transplanted upstream and downstream of the discharge. Table 4 shows average concentrations at each site. Concentrations were significantly different ($p < 0.05$) for only four elements. For three of these (Al, Se, and Ti) the mean concentration was significantly lower downstream of the discharge than upstream. The only element that was significantly higher downstream of the discharge was Ba, and the toxicology of this element is relatively unclear. One finding of interest was that tissue Cu concentrations at both stations were near or above 8 ppm (mg/kg), which is a level thought to cause sublethal effects, such as loss of copper regulation, in zebra mussels, *Dreissena polymorpha* (U.S. EPA, 2000). However, the upstream value (9 ppm) was higher than the downstream value (7.5 ppm) so these concentrations should not be attributed to the discharge.

Table 4. Elemental concentrations in tissues (mg/kg dry weight) of fatmuckets (*Lampsilis siliquoidea*, Unionidae) transplanted in the Fayette Drain upstream and downstream of the Riola Mine discharge near Georgetown, IL. Yellow shading indicates means are significantly different ($p < 0.05$).

		upstream	downstream
element		average (n = 5)	average (n = 4)
Al	mg/kg	551.6	382.2
B	mg/kg	343.3	337.8
Ba	mg/kg	464.9	537.9
Ca	mg/kg	64461.5	71738.3
Cd	mg/kg	2.7	2.7
Co	mg/kg	3.1	2.7
Cr	mg/kg	3.9	4.7
Cu	mg/kg	9.0	7.5

Fe	mg/kg	2316.0	2502.0
K	mg/kg	2637.8	2480.4
Li	mg/kg	2.1	2.0
Mg	mg/kg	2299.3	2368.5
Mn	mg/kg	5097.7	5561.5
Na	mg/kg	1718.4	1847.5
Ni	mg/kg	10.8	3.9
P	mg/kg	34506.8	38225.0
Pb	mg/kg	8.4	8.8
S	mg/kg	6120.5	6204.4
Se	mg/kg	15.0	11.4
Si	mg/kg	1448.4	1346.0
Sr	mg/kg	88.4	97.9
Ti	mg/kg	11.6	8.1
V	mg/kg	2.8	2.8
Zn	mg/kg	440.4	489.4

Conclusions—Riola Mine Discharge

Based on the data generated in this study, including water chemistry, toxicity testing, benthic macroinvertebrate sampling, and bioaccumulation studies with transplanted freshwater mussels, it appears that the discharge from the Black Beauty Riola is not having an adverse impact on the biota inhabiting the Fayette Drain. Benthic macroinvertebrate communities were more diverse downstream of the mine site and were dominated by mollusks, whereas the upstream site was dominated by rather tolerant midges (Chironomidae). None of the water or sediment samples collected were toxic to standard tests organisms. Furthermore, transplanted mussels did not accumulate significant levels of harmful metals in their tissues compared to upstream transplants after two months exposure, and their TCI values were not significantly lower than those placed upstream of the mine site. Water column and sediment toxicity testing may represent snapshots, in that if they indicate toxicity or lack thereof, it is only for the moment at which samples were collected; however, benthic macroinvertebrate surveys and mussel transplants are not. The latter two techniques, particularly benthic macroinvertebrate sampling, represent longer-term assessments of the condition of a particular site. In this case, all types of data collected were in agreement in suggesting that the discharge is not a major factor in structuring the aquatic communities of Fayette Drain.

The remaining locations did not have active discharges at the time of the study and therefore the data generated are representative of background conditions at the sites.

White County--Pattiki Coal Mine Waste Disposal Facility/Little Wabash River

A coal mine waste disposal facility near Carmi is proposed for an unnamed tributary of the Little Wabash River several miles above its confluence with the Wabash River. There is no other past or present coal mining in the watershed. At this point, the Little Wabash River is known to support populations of the State-listed threatened spike, *Elliptio dilatata*, and the federally-listed fat pocketbook, *Potamilus capax*.

The discharge tributary was located and study sites set up upstream and downstream of it. An excellent source population of mussels was located in the Little Wabash River upstream of the city of Carmi near First Street. The dominant species found in this community is *Quadrula quadrula*, the mapleleaf, and specimens were collected and caged in late August. Cages were deployed at the study sites on August 27 and retrieved on October 9, 2002. To our knowledge, the tributary receiving the discharge was dry during the mussel exposure period. However, data from this first experiment will provide insight into background metal levels and mussel condition values under low flow. I have received no information regarding whether or not the NPDES permit has been approved.

Laboratory Water Column and Sediment Toxicity Testing

Water samples collected from upstream and downstream reaches, and from the discharge itself caused no acute toxicity to *Ceriodaphnia dubia*. Likewise with whole sediment toxicity tests with *Chironomus tentans*, samples collected from upstream and downstream sites did not cause significant mortality or growth reduction to the midge larvae.

Benthic Macroinvertebrate Community Sampling

A total of 130 individuals belonging to 13 different taxa were collected at both sites (Table 5). At the upstream site, 8 taxa were identified, while 10 were identified at the downstream site. The upstream site was dominated by mayflies (Ephemeroptera) and dipterans (mostly of the family Chironomidae), whereas the downstream site was dominated by both gastropods (Pleuroceridae) and mayflies. The upstream site had four genera of mayflies, whereas only two genera were collected at the downstream site; however, more individuals from the order Ephemeroptera were collected at the downstream site.

Table 5. Complete list of organisms collected using RBP methods both upstream and downstream of the Pattiki Coal Mine Waste Disposal Facility discharge stream in the Little Wabash River near Carmi, IL. Yellow shading indicates a taxon not present at one site but collected at the other.			
		Upstream	Downstream
Family	Taxon	# of specimens	# of specimens
Annelids			

	Hirudinea	0	1
	Oligochaeta	0	2
Mollusks			
Gastropoda			
Pleuroceridae		0	38
Crustaceans			
Malacostraca/Decapoda			
Cambaridae	<i>Orconectes sp.</i>	1	0
Palaemonidae	<i>Palaemonetes kadiakensis</i>	2	3
Malacostraca/Isopoda			
Asellidae	Caecidotea	1	1
Insects			
Ephemeroptera			
Caenidae	<i>Caenis</i>	3	2
Ephemeridae	<i>Hexagenia</i>	1	0
Heptageniidae	<i>Stenonema</i>	11	34
Tricorythidae	<i>Tricorythodes</i>	1	0
Odonata			
Coenagrionidae	<i>Argia</i>	0	4
Coleoptera			
Elmidae	<i>Stenelmis</i>	0	3
Diptera			
Chironomidae		20	2
	total abundance	40	90
	total richness	8	10
	EPT richness	4	2

Caged Transplanted Mussel Study

Mussels were caged within 200 meters upstream and downstream of the tributary designated to receive the discharge. In addition, at the end of the caging experiment, 10 specimens of the same species were collected from the initial collection site (upstream of Carmi near First Street) to make the same measurements. This was done to determine if caging of mussels has an impact on their condition. As shown in table 6, there was no difference in final wet weight, length (measured as the distance from the anterior to the posterior end), or depth (measured as the distance from the dorsal to the ventral margin) at the end of the experiment between the caged mussels placed upstream and downstream of the discharge tributary. However, the mussels that were caged and transplanted had lower average weights, lengths, and depths than the “native” or un-caged mussels

collected at the end of the experiment. In spite of this finding, there was no difference among any of the groups in TCI, indicating that their overall condition was similar, despite differences in size. These results indicate that placement upstream or downstream of the discharge tributary had no impact on overall organism health. Furthermore, caging and transplanting did not have a negative impact on organism health. This is encouraging, because it indicates that when there is an environmental stressor present, its effects will not be masked by effects of taking mussels out of their natural environment and placing them in cages.

Table 6. Size and condition measurements for caged and uncaged mapleleaves, <i>Quadrula quadrula</i> , upstream and downstream of the Pattiki Coal Mine Waste Disposal Facility discharge stream in the Little Wabash River near Carmi, IL. Different capital letters indicate significant differences among means ($p < 0.05$).				
Site	Wet Weight (g)	Length (mm)	Depth (mm)	TCI*
Upstream (n = 15)	210.8 \pm 22.7 B	88.0 \pm 3.5 B	72.9 \pm 2.8 B	3.1 \pm 0.3 A
Downstream (n = 14)	188.4 \pm 21.9 B	85.2 \pm 2.2 B	69.8 \pm 2.2 B	3.3 \pm 0.5 A
Native (n = 10)	257.3 \pm 31.3 A	93.9 \pm 2.1 A	76.4 \pm 2.7 A	3.0 \pm 0.5 A
* TCI= tissue condition index, measured as dry weight of soft tissue divided by dry weight of shell, multiplied by 100.				

Generally, elemental concentrations in dried mussel tissues were similar for organisms transplanted upstream and downstream of the discharge. Table 7 shows average concentrations at each site. Concentrations were significantly different ($p < 0.05$) for only two elements, Cd and Mg. For both Cd and Mg, the mean concentration was highest in the native (uncaged) organisms. The reason for the significant decrease in these metal concentrations in caged mussels is unknown. None of the other elemental concentrations were outstanding.

Table 7. Elemental concentrations in tissues (mg/kg dry weight) of mapleleaves (<i>Quadrula quadrula</i> , Unionidae) transplanted in the Little Wabash River upstream and downstream of the Pattiki Coal Mine Waste Disposal Facility discharge stream near Carmi, IL. Yellow shading indicates means are significantly different ($p < 0.05$).				
		upstream	downstream	native
element	units	average (n = 5)	average (n = 5)	average (n = 3)
Ag	mg/kg	bdl	bdl	bdl
Al	mg/kg	32.4	33.2	33.6
As	mg/kg	2	1.9	2.1
Ba	mg/kg	472	487	500
Ca	mg/kg	29,200	31,600	31,333
Cd	mg/kg	0.4	0.5	0.8
Co	mg/kg	0.9	0.9	1.0
Cr	mg/kg	1.4	1.2	1.4

Cu	mg/kg	6.9	6.1	6.7
Fe	mg/kg	1,840	1,660	1,900
Mg	mg/kg	944	984	1,066
Mn	mg/kg	7560	8020	8,133
Na	mg/kg	940	1,029	1,193
Ni	mg/kg	7.2	3.9	4.8
P	mg/kg	24,600	25,000	26,333
Pb	mg/kg	0.3	0.3	0.3
Se	mg/kg	2	2	2
Sr	mg/kg	91.1	100.2	100.4
Zn	mg/kg	101.1	85.8	98.2

Clinton County--Monterey Coal Co. #2 Mine/Kaskaskia River

This underground coal mine located near Albers is closed, but has a coal waste facility that pumps treated ground water to avoid contamination of an aquifer, which is the principal water supply for surrounding communities. The mine has recently applied for an NPDES permit which would allow it to discharge treated water to the Kaskaskia River, just below its confluence with Shoal Creek, where it is designated as an INAI Site because it is a high-diversity (10+ species) mussel stream. No listed species are known to occur in this vicinity. No other coal mining is known to have occurred in this immediate area.

According to Mr. Jack Rickner of ExxonMobil at the Monterey #2 mine, the discharge site had not been definitively determined at the time of the study, but proposed locations include into Shoal Creek just above its confluence with the Kaskaskia River or into the Kaskaskia itself, just below Shoal Creek. I contacted the owner of the land adjacent to these sites and was granted access to the property where Shoal Creek enters the Kaskaskia River. We located a sufficient source population of mussels in the Kaskaskia drainage for use in these experiments. The site was ~4 miles northwest of Tuscola, IL, at county road 1450. The most abundant species at this site is *Amblema plicata*, the threeridge, and we collected sufficient numbers for the experiment. Cages were deployed on September 20, 2002, and removed on November 6, 2002. Mussels were also caged at the collection site near Tuscola for the same duration.

Laboratory Water Column and Sediment Toxicity Testing

Water samples collected from upstream and downstream reaches, and from the discharge itself caused no acute toxicity to *Ceriodaphnia dubia*. As with the previously described sites, whole sediment samples collected from upstream and downstream sites did not cause significant mortality or growth reduction to the midge larvae, *Chironomus tentans*.

Benthic Macroinvertebrate Community Sampling

A total of 211 individuals belonging to 8 different taxa were collected at both sites (Table 8). At the upstream site, 6 taxa were identified, 6 were identified at the downstream site as well. Both sites were dominated by hemipterans in the family Corixidae, but baetid

mayflies were present at both sites. Both sites had extremely poor habitat for most sensitive benthic macroinvertebrates as the channels were deeply cut, and the bottom substrate consisted of either shifting sands or sediment composed largely of clays.

Table 8. Complete list of organisms collected using RBP methods in Shoal Creek and the Kaskaskia River near Albers, IL.

		Shoal Creek	Kaskaskia @ Albers
Family	Taxon	# of specimens	# of specimens
Crustaceans			
Malacostraca/Decapoda			
Palaemonidae	<i>Palaemonetes kadiakensis</i>	0	2
Malacostraca/Amphipoda			
Hyalellidae	<i>Hyalella azteca</i>	1	0
Insects			
Ephemeroptera			
Baetidae	<i>Baetis</i>	19	4
Odonata			
Coenagrionidae	<i>Argia</i>	0	4
Hemiptera			
Corixidae	<i>Trichocorixa</i>	100	47
Coleoptera			
Elmidae	<i>Ancyronyx</i>	1	0
Dytiscidae	<i>Derovatellus</i>	1	0
Diptera			
Chironomidae		25	7
	total abundance	147	64
	total richness	6	6
	EPT richness	1	1

Caged Transplanted Mussel Study

Because this discharge is still in the “pending” stage, the experiment was conducted at these sites to obtain background levels for comparison when the discharge is implemented. Five cages containing three mussels each were placed in the Kaskaskia River immediately downstream of Shoal Creek, near Albers, IL, and in the mouth of Shoal Creek. Both of these sites are potential locations for the Monterey discharge. In addition, organisms were caged and placed back in the Kaskaskia River at the site at which they were collected (near Tuscola, IL). This was done to compare the responses of caged mussels at their collection site to those of organisms transplanted a large distance from the collection point. As shown in table 9, all of the caged animals had similar weights, lengths and depths at the end of the experimental period; however, significant differences ($p < 0.05$) were observed in average TCI at the end of the experiment. Specifically, organisms placed at the original collection site and in Shoal Creek, had similar average TCI, but these averages were significantly less than the average TCI of

the organisms placed in the Kaskaskia River at Albers. Because shell measurements were similar at all three sites, this difference is attributed to greater soft tissue mass in the organisms placed in the Kaskaskia at Albers. The river is much larger, in terms of discharge, at this point than it is at the collection site near Tuscola, IL, and is also much larger than Shoal Creek. It is possible that the larger discharge site is more enriched with fine particulate organic matter (FPOM) and dissolved organic carbon (Vannote et al., 1980), thus, providing a greater source of filterable food material for the caged mussels. The fact that the mussels transplanted in Shoal Creek were in similar condition to those placed back at their collection site indicates that water quality in Shoal Creek is sufficient at this time to support mussel populations. However, the physical habitat quality in Shoal Creek and in the Kaskaskia River downstream of Shoal Creek is less than optimal for freshwater unionids.

Table 9. Size and condition measurements for caged and uncaged threeridges, <i>Amblema plicata</i> , in the Kaskaskia River drainage near Albers, and Tuscola, IL. Different capital letters indicate significant differences among means ($p < 0.05$).				
Site	Weight (g)	Length (mm)	Depth (mm)	TCI*
Kaskaskia, Albers (n = 14)	358.6 ± 57.2 A	119.9 ± 4.5 A	83.0 ± 3.7 A	2.8 ± 0.9 A
Shoal Creek (n = 15)	387.6 ± 64.9 A	120.9 ± 7.7 A	83.6 ± 3.8 A	2.4 ± 0.4 B
Kaskaskia, Tuscola (n = 14)	363.7 ± 46.8 A	119.4 ± 5.9 A	82.6 ± 4.8 A	2.1 ± 0.3 B
* TCI= tissue condition index, measured as dry weight of soft tissue divided by dry weight of shell, multiplied by 100.				

Of the 19 elements measured in the mussel tissues from these sites, 15 were highest in the mussels caged at their original collection location near Tuscola, and in the case of 10 of these, concentrations were significantly higher ($p < 0.05$) at the Tuscola location than in the Kaskaskia River near Albers location (Table 10). Metals in tissues of mussels caged in Shoal Creek were generally lower than those in the Kaskaskia Tuscola location, but often not significantly lower. It appears that being caged at the Clinton County locations for two months allowed the mussels to depurate some of the metals contained in their tissues before transplantation.

Table 10. Elemental concentrations in tissues (mg/kg dry weight) of threeridges (<i>Amblema plicata</i> , Unionidae) transplanted in the <i>Amblema plicata</i> , in the Kaskaskia River drainage near Albers, and Tuscola, IL. Yellow shading indicates means are significantly different ($p < 0.05$) from cells not shaded or shaded blue. Cells shaded green are not significantly different from either yellow or blues cells.				
		Shoal Ck	Kaskaskia Albers	Kaskaskia Tuscola
element		average (n = 5)	average (n = 5)	average (n = 5)
Ag	mg/kg	2	3.5	4.1
Al	mg/kg	332	138	211
As	mg/kg	3.7	5.6	7.2

Ba	mg/kg	1220	998	1380
Ca	mg/kg	76,400	65,200	87,400
Cd	mg/kg	0.7	0.5	0.6
Co	mg/kg	1.4	1.4	1.8
Cr	mg/kg	3.8	3.6	5
Cu	mg/kg	4.6	4.5	4.3
Fe	mg/kg	2,820	2,321	2,900
Mg	mg/kg	3320	2778	3,620
Mn	mg/kg	8420	6897	8,760
Na	mg/kg	1560	1,300	1,400
Ni	mg/kg	6.1	5.3	6.2
P	mg/kg	53,200	45,503	56,400
Pb	mg/kg	2.5	2.3	2.8
Se	mg/kg	3.3	2.5	3.7
Sr	mg/kg	302	254	368
Zn	mg/kg	490	432	560

Logan County--Sandra Miller Bellrose Nature Preserve/Sugar Creek

This privately owned site is designated an INAI Site (Sugar Creek-Salt Creek) high-mussel diversity stream (at least 17 species present) and has received approval as an Illinois Nature Preserve. The site is known to contain remarkably dense mussel populations in its 0.8-mile segment, but, interestingly, no State-listed species are present.

Laboratory Water Column and Sediment Toxicity Testing

Water samples collected from upstream and downstream reaches, and from the discharge itself caused no acute toxicity to *Ceriodaphnia dubia*. As with the previously described sites, whole sediment samples collected from upstream and downstream sites did not cause significant mortality or growth reduction to the midge larvae, *Chironomus tentans*.

Benthic Macroinvertebrate Community Sampling

A total of 629 individuals belonging to 24 different taxa were collected at Sugar Creek (Table 11). This site also had the highest EPT richness of any site sampled in the study, with five genera of mayflies and three genera of caddisflies. Five genera of non-unionid mollusks were also collected at this site.

Table 11. Complete list of organisms collected using RBP methods in Sugar Creek, Sandra Miller Bellrose Nature Preserve drainage, Logan Co., IL.

		Sugar Creek
Family	Taxon	# of specimens

Annelids		
	Oligochaeta	5
Mollusks		
Pelecypoda/Veneroida		
Sphaeriidae	<i>Pisidium</i>	3
Sphaeriidae	<i>Sphaerium</i>	3
Corbiculidae	<i>Corbicula</i>	45
Gastropoda		
Pleuroceridae	<i>Pleurocera</i>	11
Lymnaeidae	<i>Fossaria</i>	1
Crustaceans		
Malacostraca/Amphipoda		
Hyalellidae	<i>Hyalella azteca</i>	1
Insects		
Ephemeroptera		
Baetidae	<i>Baetis</i>	78
Isonychiidae	<i>Isonychia</i>	7
Heptageniidae	<i>Stenacron</i>	5
Heptageniidae	<i>Stenonema</i>	219
Tricorythidae	<i>Tricorythodes</i>	20
Odonata		
Calopterygidae	<i>Calopteryx</i>	3
Coenagrionidae	<i>Argia</i>	13
Gomphidae	juveniles	5
Hemiptera		
Corixidae		1
Belastomatidae	<i>Belastoma</i>	1
Trichoptera		
Hydropsychidae	<i>Ceratopsyche</i>	10
Hydropsychidae	<i>Hydropsyche</i>	20
Hydropsychidae	<i>Cheumatopsyche</i>	88
Megaloptera		
Corydalidae	<i>Corydalus</i>	1
Coleoptera		
Elmidae	<i>Stenelmis</i>	39
Diptera		
Chironomidae		49
Tipulidae	<i>Tipula</i>	1
	total abundance	629
	total richness	24
	EPT richness	8

Caged Transplanted Mussel Study

A dense population of plain pocketbooks, *Lampsilis cardium*, was located ~5 miles upstream of the Nature Preserve in Sugar Creek, ~6 miles west of McLean, IL, on Rte. 136, and organisms were transplanted to the Nature Preserve area. Mean weight, length, depth and TCI measurements are shown in table 12. Note that the TCI values for this species are much higher than the averages for those used in other locations. This is a function of the morphology of that particular species and not the area in which they were located.

Table 12. Size and condition measurements for caged plain pocketbooks, <i>Lampsilis cardium</i> , in Sugar Creek, Sandra Miller Bellrose Nature Preserve drainage, Logan Co., IL.				
Site	Weight (g)	Length (mm)	Depth (mm)	TCI*
Sugar Creek (n = 5)	324.8 ± 33.6	123.1 ± 13.4	82.1 ± 13.7	8.08 ± 1.72
* TCI= tissue condition index, measured as dry weight of soft tissue divided by dry weight of shell, multiplied by 100.				

Generally, elemental concentrations in dried mussel tissues were low, with two notable exceptions (Table 13); lead and zinc in these mussels were higher than at any other location in the entire study.

Table 13. Elemental concentrations in tissues (mg/kg dry weight) of plain pocketbooks (<i>Lampsilis cardium</i> , Unionidae) transplanted in Sugar Creek, Sandra Miller Bellrose Nature Preserve, Logan Co., IL				
element	units	Sugar Ck average (n = 5)		
Al	mg/kg	184		
As	mg/kg	bdl		
Ba	mg/kg	297		
Ca	mg/kg	51,737		
Cd	mg/kg	0.85		
Co	mg/kg	1.1		
Cr	mg/kg	1		
Cu	mg/kg	5.3		
Fe	mg/kg	865		
Mg	mg/kg	2272		
Mn	mg/kg	5860		
Na	mg/kg	1,592		
Ni	mg/kg	4.5		
P	mg/kg	33,109		
Pb	mg/kg	13.4		
Se	mg/kg	7.225		
Sr	mg/kg	102		
Zn	mg/kg	688.5		

Data for the last three locations (Little Wabash, Kaskaskia, and Sugar Creek) were generated for comparison upon conducting a similar study after discharges were put in place, so conclusions are not relevant at this time.

References

- American Public Health Association, American Water Works Association, Water Environment Federation (APHA). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
- Barbour MT, Gerritsen J, Snyder BD, and Stribling JB. 1999 Rapid bioassessment protocols for use in streams and wadeable rivers: benthic macroinvertebrates and fish. 2nd ed. EPA 444/4089-001, Washington, DC.
- Belanger SE, Farris JL, Cherry DS, and Cairns, J, Jr. 1990. Validation of *Corbicula fluminea* growth reductions induced by copper in artificial streams and river systems. Can J Fish Aquat Sci 47:904-14.
- Cherry DS, Van Hassel JH, Farris JL, Soucek DJ, and Neves RJ. 2002. Site-specific derivation of the acute copper criteria for the Clinch River, Virginia. Hum Ecol Risk Assess 8:591-601.
- Merritt RW, and Cummins KW (1996) An introduction to the aquatic insects of North America, 3rd addition. Kendall/Hunt Publishing Company, Dubuque, IA 52002.
- Naimo TJ, Damschen ED, Rada RG, and Monroe EM. 1998. Nonlethal evaluation of the physiological health of unionid mussels: methods for biopsy and glycogen analysis. J N Am Benthol Soc 17:121-128.
- Newton TJ, Monroe EM, Kenyon R, Gutreuter S, Welke KI, and Thiel PA. 2001. Evaluation of relocation of unionid mussels into artificial ponds. J N Am Benthol Soc 20:468-485.
- Pennak RW (1989) Freshwater invertebrates of the United States, 3rd edition. John Wiley & Sons, Inc. New York
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, and Cushing CE. 1980. The river continuum concept. Can J Fish Aquat Sci 37: 130-137.
- US EPA (U.S. Environmental Protection Agency). 1985. Ambient Water Quality Criteria for Copper-1984. EPA 440/5-84-031. Office of Water, Washington, DC.
- US EPA (U.S. Environmental Protection Agency). 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed. Washington D.C. EPA/600/4-90/027F, Cincinnati, OH
- US EPA (U.S. Environmental Protection Agency). 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms, 3rd ed. Washington D.C. EPA-600-4-91-002, Cincinnati, OH

US EPA (U.S. Environmental Protection Agency). 1999. National Recommended Water Quality Criteria – Correction. EPA 822-Z-99-001. Office of Water, Washington, DC.

US EPA (U.S. Environmental Protection Agency). 2001. Methods for collecting, storage, and manipulation of sediments for chemical and toxicological analyses: Technical manual. EPA 823-B-01-002. Office of Science and Technology, Washington DC.